Parasitism of Greenbug, *Schizaphis graminum*, by the Parasitoid *Lysiphlebus testaceipes* at Winter Temperatures

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ABSTRACT Functional responses by *Lysiphlebus testaceipes* (Cresson), a common parasitoid of small grain aphids, on greenbug, *Schizaphis graminum* (Rondani), were measured at seven temperatures (14, 12, 10, 8, 6, 4, and 2°C) during a 24-h period (12-h light: 12-h dark). Oviposition by *L. testaceipes* ceased at temperatures <4°C. At all experimental temperatures, a type I, rather than a type II or type III, functional response was determined to be the best fit based on coefficient of determination (r^2) values. *L. testaceipes* was observed to oviposit in greenbugs at temperatures below the developmental temperature of both the greenbug host (5.8°C) and the parasitoid itself (6.6°C). This ability to oviposit at subdevelopmental temperatures enables the parasitoid to increase the percentage of greenbugs that are parasitized while the greenbugs are unable to reproduce. The implications of these findings regarding population suppression of greenbugs are discussed.

KEY WORDS *Lysiphlebus testaceipes*, *Schizaphis graminum*, functional response, biological control, winter hardiness

Winter wheat (*Triticum aestivum* L.) is an important multipurpose cereal crop grown in the Southern Great Plains. More than 12 million acres are planted annually for grain, forage, or as a combination grain/forage crop in Oklahoma and Texas (Epplin et al. 1998, USDA 2005). In this region of the United States, winter wheat is attacked primarily by phloem-feeding cereal aphids, resulting in reduced forage and grain yields (Gerloff and Ortman 1971, Burton 1986, Niassy et al. 1987, Peters et al. 1988, Kindler et al. 2002, 2003, K.G., unpublished data). One of the most damaging of the cereal aphids commonly found attacking winter wheat is the greenbug, *Schizaphis graminum* (Rondani). Greenbug can have a large impact on wheat production. Its economic impact in Oklahoma has ranged from $0.5 to $135 million annually (Starks and Burton 1977, Webster 1995).

Greenbug populations can be suppressed below economic injury levels through the actions of aphid parasitoids such as *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) (Jones 2001, Giles et al. 2003). *L. testaceipes* is a solitary endoparasitoid whose geographic range is Nearctic, Neotropical, and Oceanic, in addition to being Paleartic because of intentional introductions (Mackauer and Starý 1967). It has been observed to attack >100 aphid species (Mackauer and Starý 1967, Starý et al. 1988, Pike et al. 2000). *L. testaceipes* has been observed to suppress greenbug populations below economic injury levels in wheat directly through mortality and indirectly by reducing reproductive potential (Spencer 1926, Eikenbary and Rogers 1974, Giles et al. 2003). Additionally, *L. testaceipes* causes aphids to drop from the plant in an attempt to avoid parasitism. Once on the ground, aphids are highly subject to desiccation and attack by other natural enemies (Losey and Denno 1998).

Because of the relatively moderate climate in Oklahoma and Texas, greenbugs and other cereal aphids are able to feed on wheat throughout fall, winter, and spring months (Elliott et al. 2003, Royer et al. 2005). Adult parasitoids have been observed actively foraging on cool sunny days in Oklahoma throughout the winter months (D.B.J., unpublished data). However, when winter temperatures are at the lower extremes commonly encountered during wheat production, little is known about the relationship between *L. testaceipes* and its greenbug host.

Our previous work on *L. testaceipes* attack rates were based on assumptions by integrated pest management (IPM) practitioners (Patrick and Boring 1990, Royer et al. 1998). They suggested that parasitoids could not suppress greenbug populations at cool temperatures such as <14°C because parasitoid development was delayed relative to their aphid hosts. Indeed, studies showing lower developmental thresholds for greenbug (5.8°C; Walgenbach et al. 1988) versus *L. testaceipes* (6.6°C; Royer et al. 2001) and dramatic reductions in attack rates by *L. testaceipes* as temperatures were decreased to 14°C (Jones

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et al. 2003) support this assumption. However, recent field observations on the suppression of greenbug by *L. testaceipes* during cold winter months suggest that adult parasitoids are actively foraging at temperatures below greenbug developmental thresholds and effectively preventing populations from increasing (Jones 2001, Giles et al. 2003).

The primary objective of this study was to measure the 24-h functional response of *L. testaceipes* on greenbugs infesting winter wheat at 14°C and repeat these measurements at progressively colder temperatures until *L. testaceipes* failed to parasitize greenbug hosts. Additionally, we studied the relationship between temperature and the proportion of *L testaceipes* females that oviposited at each temperature.

Materials and Methods

Greenbug and Parasitoid Colonies. Biotype "E" greenbugs were obtained from colonies maintained at the USDA–ARS Plant Science and Water Conservation Research Laboratory at Stillwater, OK; some were established on grain sorghum (cultivar SG-925), and others were established on wheat (cultivar 2137) grown in a fritted clay and sphagnum moss mixture. Insect colonies and all plants were kept inside double-walled fine mesh cages located within a climate-controlled greenhouse (≈22°C). The double-walled cages prevented contamination of colonies by feral greenbugs and parasitoids while permitting ample airflow. Fresh plants were rotated as needed into cages housing colonies.

Three parasitoid colonies were maintained at 22 ± 1°C and a photo-period of 12:12 (L:D) in double-walled fine mesh cages in growth chambers. *L. testaceipes* was isolated from specimens collected in Caddo County, OK, in the spring of 2003 (40 *L. testaceipes* adults isolated from greenbug mummies). Using subsamples of parasitoid offspring, we verified the parasitoids as *L. testaceipes* by keys (Pike et al. 1997) and polymerase chain reaction (PCR) analysis (Chen et al. 2002, Jones et al. 2005). Pots of grain sorghum infested by greenbugs were placed in the colonies every 3–4 d to maintain a steady supply of parasitoids. Parasitoid colonies were maintained on grain sorghum because wheat stock plants succumbed relatively quickly to greenbug feeding damage.

Functional Response Evaluations. Wheat seed (cultivar 2137) was planted in 5-cm-diameter by 20-cm-tall Ray Leach "conetainers" (Stuewe & Sons, Corvallis, OR). When plants were ≈30 cm tall (≈3–4 wk), they were thinned to two similar sized tillers that were threaded through a 0.6-cm-diameter hole in a 5-cm-diameter by 0.6-cm-thick circular Plexiglas disk. The disk was fitted into the conetainer at soil level and cotton filled up the remaining area of the hole to create a sealed experimental arena floor that prevented access to the soil. A 5-cm-diameter by 30-cm-tall clear acetate tube cage with two 5-cm holes covered with fine mesh polyester netting in the sides (to allow ventilation) was fitted around the top of the conetainer. The top of each tubular cage was also covered with netting that was held in place by a rubber band. Greenbugs from the colonies reared on wheat were introduced by placing second and third instars on wheat tillers in each conetainer with a fine brush. By only using similar-aged greenbugs, possible complicating factors such as host age preference by the wasps were avoided. We established greenbugs in conetainers at densities that ranged from 5 to 80 greenbugs per conetainer at each of the following seven temperatures in growth chambers: 2, 4, 6, 8, 10, 12, and 14°C. Because greenbugs are somewhat fragile, mortality from handling made it difficult to establish a predetermined density of greenbugs. Additionally, pedogenesis, reproduction by nymphs, occurs in ≈2% of immature greenbugs (Wood and Starks 1975). Because of these difficulties, we targeted four density ranges (≤20, 21–40, 41–60, and 61–80 greenbugs/conetainer) at each experimental temperature. This ensured a sufficient range of densities necessary to describe the functional response (Jones et al. 2003). Actual numbers of greenbugs in each conetainer were determined when greenbugs were later dissected. Greenbugs were allowed to acclimate at each temperature for 4 h before parasitoids were introduced. A minimum of six conetainer replicates were evaluated at each temperature and density range. Because all temperatures and densities could not be run at the same time, temperatures and density combinations were run in a random order.

To have naïve parasitoids that developed in greenbugs reared on wheat, conetainers of wheat were infested with 25–35 third-instar and older greenbugs from the wheat stock colony. By limiting the number of greenbugs, the fitness of emerging parasitoids was not influenced by plant health (Fuentes-Granados et al. 2001). These greenbugs were allowed to feed overnight, after which five male/female pairs of *L. testaceipes* parasitoids were released into each conetainer cage. Parasitized greenbugs were allowed to develop into mummies, after which they were removed from the colony and placed individually into 1.5-ml microcentrifuge vials. These isolated mummies were allowed to develop until they emerged as adults. On emergence, the parasitoids were sexed and paired to allow mating. Only parasitoids that had emerged on the day of the experiment were used in that day's work. Parasitoids destined for evaluation were placed into the growth chamber to acclimatize at each experimental temperature for 4 h before being released into designated conetainers with greenbugs.

Parasitoids were released as a mated pair in each experimental conetainer during the dark cycle. The lights came on the next morning at 0600 hours and turned off 12 h later at 1800 hours, after which both parasitoids in each conetainer were removed, and their survival was recorded. If a female wasp did not survive, data from that conetainer were not used. Survival of the male wasp was noted, but did not influence whether data were discarded. During the 24-h period that the parasitoids were exposed to greenbugs, they were only active during the 12-h light period and were quiescent when lights were off (D.B.J., unpublished

data). After the removal of parasitoids, conetainers were placed in a chamber at 22°C for 2–3 d to allow parasitoid eggs to develop into larvae before dissections were attempted. Subsequently, conetainers were held at 5°C to arrest parasitoid development, until all greenbugs were dissected. Eggs of aphid parasitoids are quite difficult to detect, thus delaying dissections until after hatching greatly improved accuracy of data (Hofsvang and Hågvar 1978, van Steenis 1993, Jones et al. 2003). Encapsulation could hinder accuracy, but encapsulation of *L. testaceipes* by *S. graminum* has yet to be observed (D.B.J., unpublished data).

Dissections were performed in an aqueous solution of 2% saline (NaCl) and 1% dishwashing detergent to act as a surfactant. Greenbugs were dissected by grasping the head with a pair of fine forceps and "pricking" the caudal region with a second pair of fine forceps, opening the body cavity. Contents were gently squeezed from the greenbug into the dissecting solution and examined for the presence of parasitoid larvae. Although *L. testaceipes* is solitary, superparasitism frequently occurs (Jones et al. 2003). Therefore, numbers of larvae present in each greenbug and the total numbers of greenbugs per experimental unit (conetainer cage) dissected were recorded. Although some eggs may fail to hatch, the total number of parasitoid larvae present was assumed to be equal to the total number of eggs laid per female in 24 h (Hofsvang and Hågvar 1978, van Steenis 1993).

Statistical Analyses. All statistical analyses were performed using PC SAS version 8.2 (SAS Institute 1999) at a significance level of $P = 0.05$. Coefficients of determination (r^2 values) were calculated using PROC NLIN to determine which functional response model (type I, II, or III) best described the number of greenbugs parasitized at each temperature over the range of host densities. The following models were evaluated:

Type I: $N_A = aTN$ (Holling 1959a)

Type II: $N_A = aTN/(1 + aT_hN)$ (Holling 1959b)

Type III: $N_A = N\{1 - \exp[-aT/(1 + aT_hN)]\}$ (Hassell et al. 1977)

In these models, N_A is the number of hosts parasitized, N is the initial host density, T is the time available for searching during the experiment, a is the instantaneous attack rate, and T_h is the amount of time the parasitoid spent handling the host. For the type I models, the parameter a, along with the parameters a and T_h for the type II and type III models, were estimated using PROC NLIN (Donnelly and Phillips 2001, Jones et al. 2003). Although these parameters can be measured by observation (Mills and Gutierrez 1999), it was not practical to do so in this experiment.

Typically, functional responses are calculated for only those predators or parasitoids that actually attack their prey or host and are perceived of as normally functioning animals. However, in this paper, we also estimated functional response for all of the female parasitoids including those that remained alive but did not oviposit. We did this because our observations indicated that as temperatures decreased the proportion of parasitoids that oviposited decreased as well. This decrease in the proportion of ovipositing parasitoids may help to describe *L. testaceipes* biology at suboptimal temperatures and the resulting dynamics with greenbug populations in field situations. While including nonparasitizing parasitoids in the analyses was not typical of functional response models, these nonovipositing parasitoids are viable, potential attackers of aphids that may only need warmer temperatures to become active.

Voucher Specimens. Voucher specimens of *L. testaceipes* adults and mummies and *S. graminum* adults were deposited in the Department of Entomology and Plant Pathology museum at Oklahoma State University in Stillwater.

Results and Discussion

Parasitism at Low Temperatures. Previous work by Jones et al. (2003) suggested that *L. testaceipes* should be able to oviposit at temperatures <14°C. This experiment confirmed that assumption as we observed that 23.3% of *L. testaceipes* females assayed successfully oviposited at 4°C (Fig. 1). This minimum temperature is very close to observations by Hunter and Glenn (1909), who, with limited observations, reported that *L. testaceipes* could oviposit at 3.3°C. This result also compares well with field observations that *L. testaceipes* can be active during typical Oklahoma winter temperatures (Pomeroy and Brun 1999, Giles et al. 2003).

These observations are interesting because *L. testaceipes* is actively ovipositing at temperatures below its developmental threshold of 6.6°C (Royer et al. 2001) and below the developmental temperature threshold of its greenbug host (greenbug developmental threshold = 5.8°C; Walgenbach et al. 1988). Provided adult females are present in wheat fields during the winter, this ability to oviposit at temperatures below the developmental threshold of the host enables the parasitoid to effectively increase its population levels (within greenbug hosts) while the host cannot in-

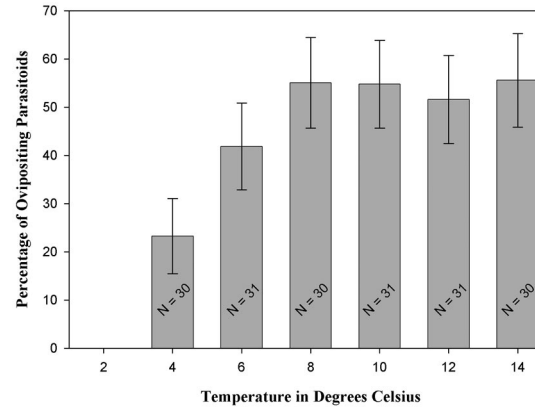

Fig. 1. Bar graph with SE bars, showing the percent of *L. testaceipes* females who successfully oviposited in greenbug, *S. graminum*, over 24 h (12:12 L:D) at 2, 4, 6, 8, 10, 12, and 14°C.

Table 1. Coefficients of determination for functional response regression models for *L. testaceipes* at 2, 4, 8, 10, 12, and 14°C (12:12 L:D) on greenbugs

Parasitoid species[a]	Temperature (±1°C)	Type I (r^2)	Type II (r^2)	Type III (r^2)
L. testaceipes	2[b]	NA	NA	NA
	4[b]	0.150	0.150	0.150
	6[b]	0.335	0.335	0.335
	8[b]	0.461	0.461	0.461
	10[b]	0.344	0.398	0.398
	12[b]	0.283	0.308	0.308
	14[b]	0.405	0.406	0.406
	2[c]	NA	NA	NA
	4[c]	0.655	0.669	0.669
	6[c]	0.483	0.524	0.524
	8[c]	0.625	0.625	0.625
	10[c]	0.667	0.719	0.720
	12[c]	0.480	0.523	0.524
	14[c]	0.721	0.730	0.730

[a] *Lysiphlebus testaceipes* host densities ranged from 5 to 80 greenbugs per experimental unit. Type I, II, and III functional response equations were evaluated using SAS PROC NLIN to generate coefficients of determination (r values), indicating best fit.

[b] Functional response r^2 values calculated using all parasitoids at that temperature.

[c] Functional response r^2 values calculated using only those parasitoids that oviposited at that temperature.

NA, not applicable.

crease its population. As experimental temperatures increased, so did the percentage of ovipositing females (Fig. 1). However, percentages were similar at 8–14°C. As environmental temperature increased above the developmental threshold for greenbugs, several factors including numerical and functional responses influence the dynamics between *L. testaceipes* and its host.

Functional Response Calculations. When considering all experimental parasitoids including those that did not oviposit, because of large amounts of variation in attack rates between individual parasitoids, we were unable to determine which functional response model (type I, II, or III) provided the best fit at 4, 6, and 8°C (Table 1). None of the models provided a best fit because the coefficients of determination (r^2) were only 0.15 for each model at 4°C, 0.34 for each model at 6°C, and 0.46 for each model at 8°C. At 10, 12, and 14°C, a type II functional response model better described the relationship between greenbug density and the attack rate of *L. testaceipes* than a type I model, but was indistinguishable from a type III model. However, the r^2 values for the type II and type III models were only marginally better than for a type I model (Table 1). Because of little to no differences in r^2 values, we used the linear type I model for making comparisons between temperatures. Comparisons of instantaneous attack rates (a) estimated from type I functional response models (a type I model is the simplest of the three models) revealed that the 4°C functional response model was not significantly different from the 6°C model but was significantly different (lower) than the models for all other experimental temperatures (Table 2). When instantaneous attack rates (a) were calculated for the type II models, no significant differences were observed (Table 2). Handling time (T_h) estimates were also generated for the type II models; however, no significant differences were observed among temperatures.

When those parasitoids that did not oviposit were removed from the calculations, the functional response coefficients of determination improved considerably. Again the r^2 values were only marginally better for type II or type III functional response models over the coefficient of determination values for a type I model. Instantaneous attack rates (a) estimated from type I functional response models revealed that the 4 and 6°C models were significantly different from the 14°C model but were not significantly different from the 8, 10, and 12°C models. Conversely, the 14°C model was also not significantly different from the 8,

Table 2. Estimates of instantaneous attack rates (a) for all *L. testaceipes* females evaluated calculated from experimental data fit to type I and II functional response models

Functional response model	Temperature (°C)	Instantaneous attack rate (a ± SE)[a]	Handling time (Th ± SE)[a]
Type I	2	NA	NA
	4	0.02 ± 0.01a	NA
	6	0.08 ± 0.02ab	NA
	8	0.22 ± 0.04b	NA
	10	0.12 ± 0.03b	NA
	12	0.14 ± 0.05b	NA
	14	0.19 ± 0.05b	NA
Type II	2	NA	NA
	4	0.16 ± 0.66a	0.67 ± 0.70a
	6	0.08 ± 0.02a	0.00 ± 0.00a
	8	0.22 ± 0.04a	0.00 ± 0.00a
	10	0.58 ± 1.16a	0.10 ± 0.07a
	12	0.35 ± 0.55a	0.06 ± 0.08a
	14	0.21 ± 0.18a	0.01 ± 0.06a

[a] a and Th estimated by PROC NLIN.

Means sharing the same letter are not significantly different at $\alpha = 0.05$.

NA, not applicable.

Table 3. Estimates of instantaneous attack rates (a) for *L. testaceipes* females that successfully oviposited, calculated from experimental data fit to type I and II functional response models

Functional response model	Temperature (°C)	Instantaneous attack rate (a ± SE)[a]	Handling time (Th ± SE)[a]
Type I	2	NA	NA
	4	0.10 ± 0.03a	NA
	6	0.12 ± 0.03a	NA
	8	80.29 ± 0.06ab	NA
	10	0.22 ± 0.04ab	NA
	12	0.24 ± 0.07ab	NA
	14	0.34 ± 0.06b	NA
Type II	2	NA	NA
	4	0.25 ± 0.41a	0.10 ± 0.12a
	6	0.61 ± 1.00a	0.09 ± 0.06a
	8	0.29 ± 0.17a	0.00 ± 0.00a
	10	0.87 ± 1.20a	0.05 ± 0.03a
	12	0.68 ± 1.07a	0.04 ± 0.04a
	14	0.48 ± 0.35a	0.01 ± 0.02a

[a] a and Th estimated by PROC NLIN.

Means sharing the same letter are not significantly different at $\alpha = 0.05$.

NA, not applicable.

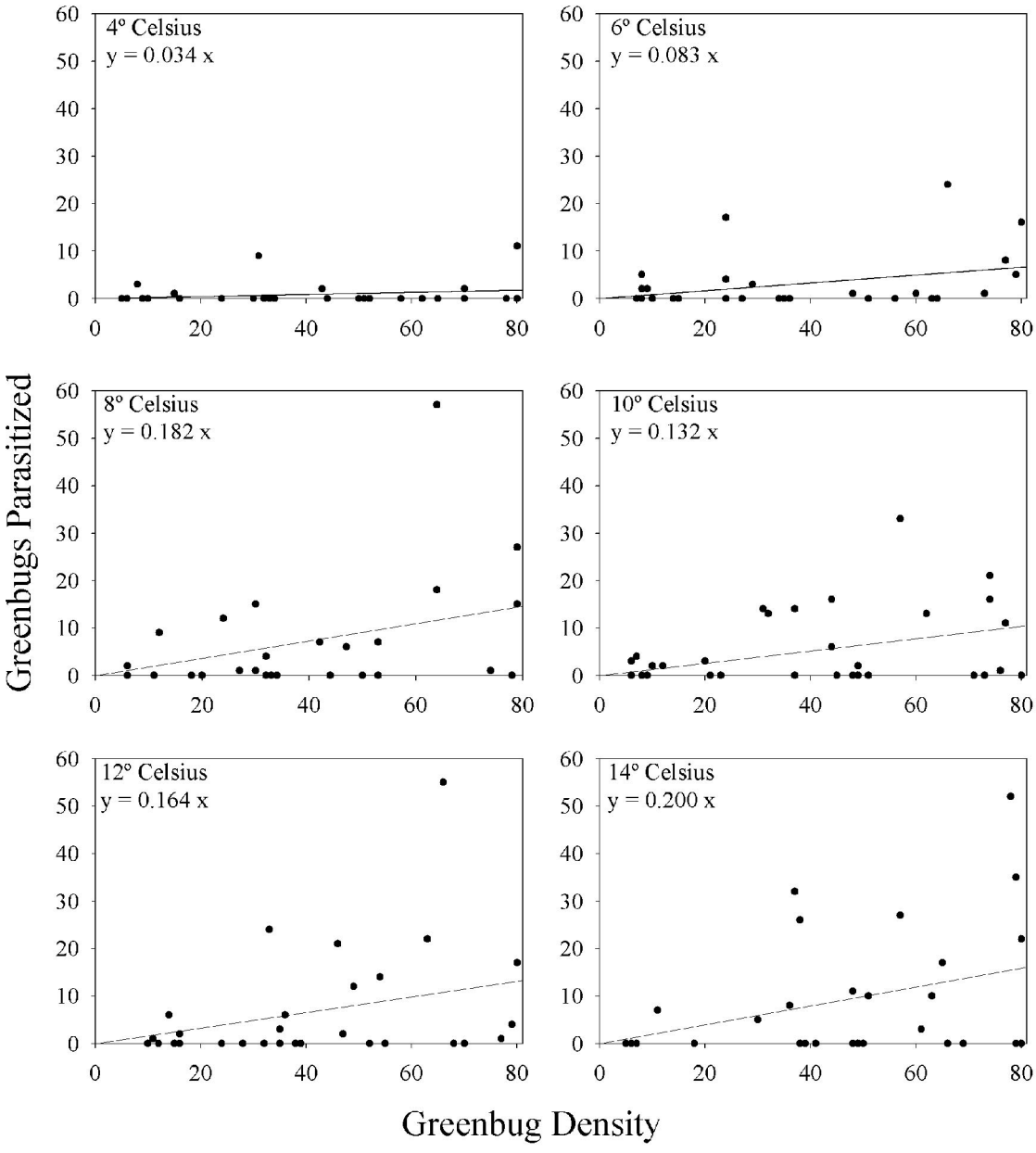

Fig. 2. Scatter plots with linear regression trend lines (type I functional response) for *L. testaceipes* attack rates (excluding those parasitoids that did not oviposit) at 4, 6, 8, 10, 12, and 14°C (12:12 L:D). The 2°C scatter plot was omitted because no oviposition occurred.

10, and 12°C models (Table 3). When instantaneous attack rates (a) and handling time estimates (T_h) were calculated for the type II models, no significant differences were observed (Table 3).

Whether we considered only *L. testaceipes* females that oviposited or all of the experimental parasitoids, type II and type III models provided only a slightly improved fit with regard to r^2 values (Table 1). Additionally, the extremely small handling times observed seem to be biologically insignificant and provide little predictive power when describing the relationship between greenbug density and attack rates of *L. testaceipes.* At temperatures >14°C, a type III functional response model provided the best fit for describing the attack rate of *L. testaceipes* on greenbug at increasing host densities (Jones et al. 2003). However, we were unable to determine which functional response model best describes changes in *L. testaceipes* attack rate on greenbug at temperatures <14°C. Again using the simple linear model (type I)

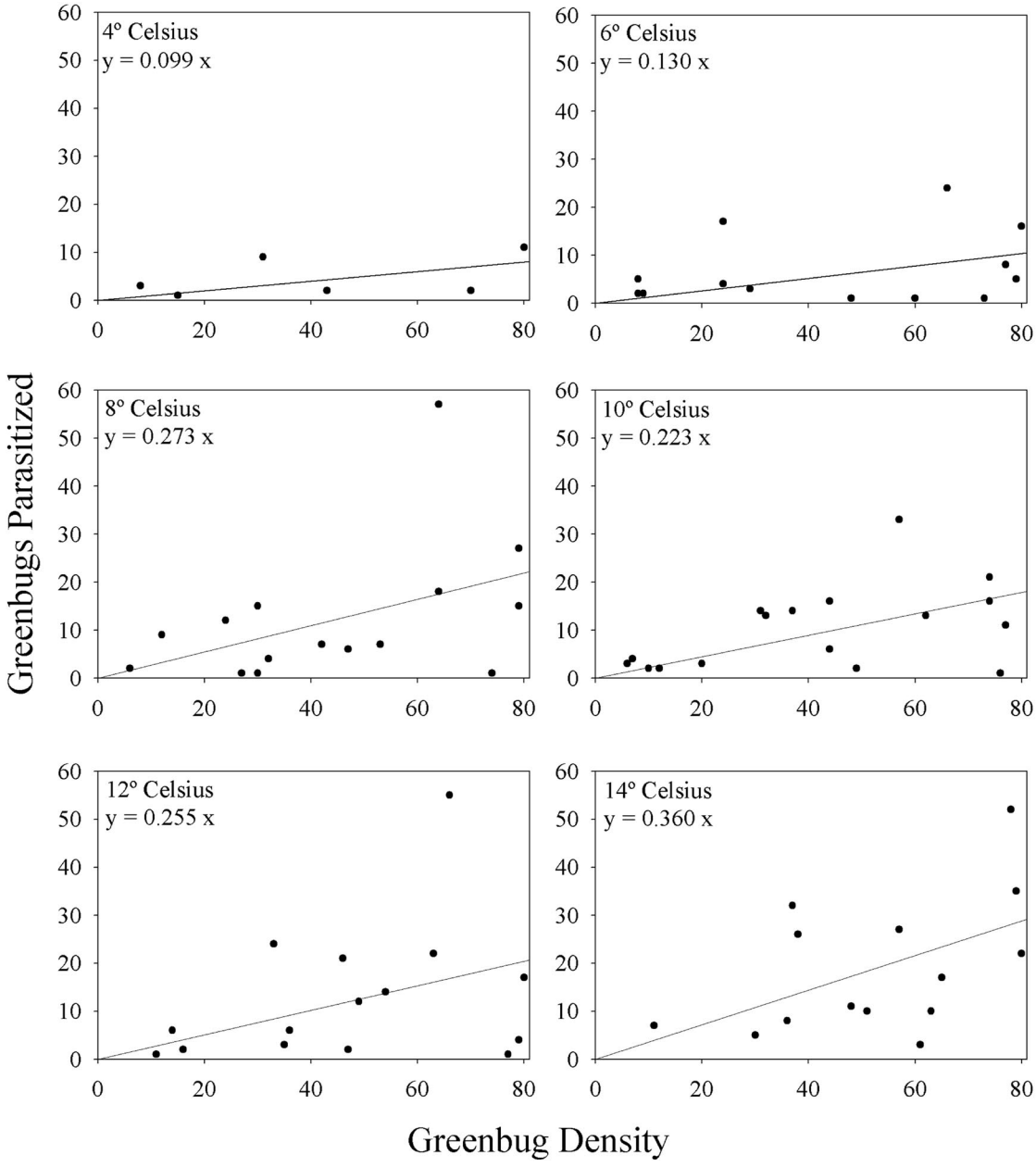

Fig. 3. Scatter plots with linear regression trend lines (type I functional response) for *L. testaceipes* attack rates (including those parasitoids that did not oviposit) at 4, 6, 8, 10, 12, and 14°C (12:12 L:D). The 2°C scatter plot was omitted because no oviposition occurred.

for comparisons of *L. testaceipes* attack rates between temperatures, we determined that the slopes describing these attack rates are quite similar between 8 and 14°C; however, the slope is significantly different at 4°C (Figs. 2 and 3).

Implications for Winter Ecology of *L. testaceipes*. A functional response is defined as the change in attack rate of a parasitoid or a predator exposed to increasing host densities per defined unit of time (Solomon 1949). The results of this experiment show that, at temperatures <14°C, functional response models are poor predictors of *L. testaceipes* attack rates (Fig. 2). Despite this poor predictive ability of the models, we observed suppression of greenbug and other cereal aphid populations by *L. testaceipes* during the cold winter months in Oklahoma (Jones 2001, Giles et al. 2003).

Our study adds additional information toward understanding why *L. testaceipes* can be such an effective natural enemy in the Southern Great Plains during

winter months. When *L. testaceipes* is present in winter wheat fields during the mild autumns (>14°C during August to November), this parasitoid is able to contribute toward suppression of aphid populations by a combination of (1) a high attack rate (Jones 2001, Giles et al. 2003), (2) sterilization of attacked aphids (Spencer 1926, Hight et al. 1972, Eikenbary and Rogers 1974), (3) dislodgment of aphids from the plant (Losey and Denno 1998), and (4) its reproductive (numerical) response from attacked aphids (Giles et al. 2003). These factors are also important contributions toward aphid suppression during the mild spring months from February to May. During December and January, when temperatures are often <14°C, the reproductive response may be relatively unimportant. As temperatures continue to drop, a developmental advantage occurs with greenbugs (5.8°C; Walgenbach et al. 1988) that have a developmental threshold lower than *L. testaceipes* (6.6°C; Royer et al. 2001).

Providing that temperatures do not drop below the threshold for aphid development, aphids should continue to numerically increase at rates higher than *L. testaceipes.* Despite weak functional response relationships at cool temperatures, a significant proportion of female *L. testaceipes* parasitoids continue to attack greenbugs as temperatures decrease below developmental thresholds for both the host greenbug and the parasitoid (Fig. 1). Under these low temperature conditions, *L. testaceipes* adult females can continue to parasitize, sterilize, and dislodge greenbugs without significant development or reproduction by the host. Additionally, *L. testaceipes* are longer lived at colder temperatures and are able to inflict mortality for extended periods of time (up to 3 wk; D.B.J., unpublished data).

These characteristics of *L. testaceipes* could enable the parasitoid to keep its population expanding (relative to aphid hosts) even when the weather is not optimal for reproduction and subsequent development. Eventually adult parasitoids will die and/or exhaust their egg load during this period. However, the parasitoid progeny within their greenbug hosts are in a state of reduced or arrested development. The progeny is alive and able to develop once temperatures increase (Archer et al. 1973, 1974, Royer et al. 2001). Indeed, we collected apparently healthy greenbugs from winter wheat fields in January and early February, which were all or mostly all parasitized (Giles et al. 2003).

Understanding interactions between greenbugs and *L. testaceipes* during cold winter weather in the Southern Great Plains requires information on the influence of decreasing temperatures on parasitoid ecology/biology. The observed low r^2 values for functional response models evaluated in our study indicate that attack rates at temperatures <14°C would be difficult to predict. The actual within-field interactions between *S. graminum* and *L. testaceipes* during the winter will depend on multiple factors including the relationship between microclimate temperatures and activity (attack by *L. testaceipes*), development, and reproduction. A future model with all of these factors will allow us to validate field collected population dynamics data.

Acknowledgments

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References Cited

Archer, T. L., C. L. Murray, R. D. Eikenbary, K. J. Starks, and R. D. Morrison. 1973. Cold storage of *Lysiphlebus testaceipes* mummies. [*Schizaphis graminum*, grain pest control]. Environ. Entomol. 2: 1104–1108.

Archer, T. L., C. L. Murray, R. D. Eikenbary, and R. L. Burton. 1974. Cold storage of *Lysiphlebus testaceipes* adults. Environ. Entomol. 3: 557–558.

Burton, R. L. 1986. Effect of greenbug (Homoptera: Aphididae) damage on root and shoot biomass of wheat seedlings. J. Econ. Entomol. 79: 633–636.

Chen, Y., K. L. Giles, M. E. Payton, and M. H. Greenstone. 2002. Molecular evidence for a species complex in the genus Aphelinus (Hymenoptera: Aphelinidae), with additional data on aphidiine phylogeny (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 95: 29–34.

Donnelly, B. E., and T. W. Phillips. 2001. Functional response of *Xylocoris flavipes* (Hemiptera: Anthocoridae): effects of prey species and habitat. Environ. Entomol. 30: 617–624.

Eikenbary, R. D., and C. E. Rogers. 1974. Importance of alternate hosts in establishment of introduced parasites. Tall timbers conference on ecological animal control by habitat management, 28 February–1 March, Tallahassee, FL.

Elliott, N. C., K. L. Giles, T. A. Royer, S. D. Kindler, F. L. Tao, D. B. Jones, and G. W. Cuperus. 2003. Fixed precision sequential sampling plans for the greenbug and bird-cherry-oat aphid (Homoptera: Aphididae) in winter wheat. J. Econ. Entomol. 96: 1585–1593.

Epplin, F. M., R. R. True, and E. G. Krenzer, Jr. 1998. Practices used by Oklahoma wheat growers by region. Oklahoma: Current Farm Economics. 7: 14–24.

Fuentes-Granados, R. G., K. L. Giles, N. C. Elliott, and D. R. Porter. 2001. Assessment of greenbug-resistant wheat germplasm on *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) oviposition and development in greenbug over two generations. Southwest. Entomol. 26: 187–194.

Gerloff, E. D., and E. E. Ortman. 1971. Physiological changes in barley induced by greenbug feeding stress. Crop Sci. 11: 174–176.

Giles, K. L., D. B. Jones, T. A. Royer, N. C. Elliott, and S. D. Kindler. 2003. Development of a sampling plan in winter wheat that estimates cereal aphid parasitism levels and predicts population suppression. J. Econ. Entomol. 96: 975–982.

Hassell, M. P., J. H. Lawton, and J. R. Beddington. 1977. Sigmoid functional responses by invertebrate predators and parasitoids. J. Anim. Ecol. 46: 249–262.

Hight, S. C., R. D. Eikenbary, R. J. Miller, and K. J. Starks. 1972. The greenbug and *Lysiphlebus testaceipes.* Environ. Entomol. 1: 205–209.

Hofsvang, T., and E. B. Hågvar. 1978. Larval morphology and development of *Aphidius colemani* Viereck and *Ephedrus cerasicola* Starý (Hym., Aphidiidae). Norw. J. Entomol. 25: 1–8.

Holling, C. S. 1959a. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. Can. Entomol. 91: 293–320.

Holling, C. S. 1959b. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91: 385–398.

Hunter, S. J., and P. A. Glenn. 1909. The greenbug and its enemies. Univ. Kans. Sci. Bull. 9: 221.

Jones, D. B. 2001. Natural enemy thresholds for greenbug, *Schizaphis graminum* Rondani, on winter wheat. MS thesis, Oklahoma State University, Stillwater, OK.

Jones, D. B., K. L. Giles, R. C. Berberet, T. A. Royer, N. C. Elliott, and M. E. Payton. 2003. Functional responses of an introduced parasitoid and an indigenous parasitoid on greenbug at four temperatures. Environ. Entomol. 32: 425–432.

Jones, D. B., K. L. Giles, Y. Chen, and K. A. Shufran. 2005. Estimation of Hymenopteran parasitism in cereal aphids using molecular markers. J. Econ. Entomol. 98: 217–221.

Kindler, S. D., N. C. Elliott, K. L. Giles, and T. A. Royer. 2003. Economic injury level for the greenbug, *Schizaphis graminum,* in Oklahoma winter wheat. Southwest. Entomol. 28: 163–166.

Kindler, S. D., N. C. Elliott, T. A. Royer. K. L. Giles, F. Tao, and R. Fuentes. 2002. Effect of greenbugs on winter wheat yield. J. Econ. Entomol. 95: 89–95.

Losey, J. E., and R. F. Denno. 1998. The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. Ecol. Entomol. 23: 53–61.

Mackauer, M., and P. Starý. 1967. World Aphidiidae: Hym. Ichneumonoidea. Le Francois, Paris, France.

Mills, N. J., and A. P. Gutierrez. 1999. Biological control of insect pests: a tritrophic perspective, pp 89–102. *In* B. A. Hawkins and H. V. Cornell (eds.), Theoretical approaches to biological control. Cambridge University Press, Cambridge, UK.

Niassy, A., J. D. Ryan, and D. C. Peters. 1987. Variations in feeding behavior, fecundity, and damage of biotypes B and E of *Schizaphis graminum* on three wheat genotypes. Environ. Entomol. 16: 1163–1168.

Patrick, C. D., and E. P. Boring, III. 1990. Managing insect and mite pests of Texas small grains. Texas Agricultural Extension Service, College Station, TX.

Peters, D. C., D. Kerns, G. Puterka, and R. W. McNew. 1988. Feeding behavior, development, and damage by biotypes B, C and E of *Schizaphis graminum* in wintermalt and post barley. Environ. Entomol. 17: 503–507.

Pike, K. S., P. Stary, T. Miller, D. Allison, L. Boydston, G. Graf, and R. Gillespie. 1997. Small-grain aphid parasitoids (Hymenoptera: Aphelinidae and Aphidiidae) of Washington: distribution, relative abundance, seasonal occurrence, and key to known North American species. Environ Entomol. 26: 1299–1311.

Pike, K. S., P. Starý, T. Miller, G. Graf, D. Allison, L. Boydston, and R. Miller. 2000. Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of northwest USA. Proc. Entomol. Soc. Wash. 102: 688–740.

Pomeroy, J. W., and E. Brun. 1999. Physical properties of snow, pp. 45–118. *In* H. G. Jones, J. W. Pomeroy, D. A. Walker, and R. Hoham (eds.), Snow ecology. Cambridge University Press, Cambridge, UK.

Royer, T. A., K. L. Giles, and N. C. Elliott. 1998. Small grain aphids in Oklahoma. Oklahoma Cooperative Extension Service, Stillwater, OK.

Royer, T. A., K. L. Giles, and N. C. Elliott. 2001. Developmental response of three geographic isolates of *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae) to temperatures. Environ. Entomol. 30: 637–641.

Royer, T. A., K. L. Giles, T. Nyamanzi, R. M. Hunger, E. G. Krenzer, N. C. Elliott, S. D. Kindler, and M. Payton. 2005. Economic evaluation of the effects of planting date and application rate of imidacloprid for management of cereal aphids and barley yellow dwarf in winter wheat. J. Econ. Entomol. 98: 95–102.

SAS Institute. 1999. PC SAS version 8.2. SAS Institute, Cary, NC.

Spencer, H. 1926. Biology of the parasite and hyper parasites of aphids. Ibid 19: 119–157.

Solomon, M. E. 1949. The natural control of animal populations. J. Anim. Ecol. 18: 1–45.

Starks, K. J., and R. L. Burton. 1977. Preventing greenbug [*Schizaphis Graminum*] outbreaks [small grains and sorghum]. Washington, DC, USDA-ARS. 11 p.

Starý, P., J. P. Lyon, and F. Leclant. 1988. Biocontrol of aphids by the introduced *Lysiphlebus testaceipes* (Cress.) (Hym., Aphidiidae) in Mediterranean France. J. Appl. Entomol. 105: 74–78.

U.S. Department of Agriculture. 2005. National agricultural statistics survey (http://www.usda.gov/nass/).

van Steenis, M. J. 1993. Intrinsic rate of increase of *Aphidius colemani* Vier. (Hym., Braconidae), a parasitoid of *Aphis gossypii* Glov. (Hom., Aphididae), at different temperatures. J. Appl. Entomol. 116: 192–198.

Walgenbach, D. D., N. C. Elliot, and R. W. Kieckhefer. 1988. Constant and fluctuating temperature effects on developmental rates and life table statistics of the greenbug. J. Econ. Entomol. 81: 501–507.

Webster, J. A. 1995. Economic impact of the greenbug in the western United States: 1992–1993. Great Plains Agricultural Council, Stillwater, OK.

Wood, E. A., and K. J. Starks. 1975. Incidence of paedogenesis in the greenbug. Environ. Entomol. 4: 1001–1002.

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